

Helminth Parasites of the Eastern Box Turtle,
Terrapene carolina carolina (L.)
(Testudines: Emydidae), in North Carolina

MICHAEL D. STUART¹ AND GROVER C. MILLER

Department of Zoology, North Carolina State University
Raleigh, North Carolina 27695-7617

ABSTRACT—We examined 117 eastern box turtles, *Terrapene carolina carolina*, for helminth parasites. Nine species (two trematodes, six nematodes, and one acanthocephalan) were recovered, and 39% of the turtles were infected with three–five species of parasites. Infection rates were as follows: *Oswaldocruzia* sp. (82.9%), *Spironoura affinis* (76.1%), *Telorchis robustus* (29.9%), *Cosmocercoides dukae* (20.5%), *Aplectana* sp. (6.0%), *Brachycoelium salamandrae* (2.6%), *Physaloptera* sp. (2.6%), *Serpinema* (= *Camallanus*) *microcephalus* (0.9%), and *Macracanthorhynchus ingens* (0.9%). Ulcerations of the stomach mucosa harbored larval *Spironoura affinis*. The presence of *Spironoura affinis*, *Telorchis robustus*, and *Serpinema microcephalus* suggests a close phylogenetic relationship of *Terrapene* to other emydid turtles. The other helminth species are normally found in amphibians and might represent parasites acquired in the turtle's evolutionary transition from an aquatic to a terrestrial lifestyle.

The box turtle, *Terrapene carolina* (L.), is found throughout the eastern United States. This small, terrestrial turtle has been studied more thoroughly than most reptile species, perhaps because of its ubiquity and innocuousness. The wealth of our knowledge on diet, habitat preference, and behavior makes the box turtle an excellent model for investigating parasite–host interactions (Stuart and Miller 1987).

This study was initiated to determine the following: (1) helminth intensity and prevalence in box turtles in North Carolina; (2) correlations, if any, of host age and sex with helminth intensity and prevalence; (3) similarity of helminth fauna in host specimens from North Carolina and elsewhere in the United States; and (4) helminth infection patterns in relation to box turtle behavior and dietary habits.

MATERIALS AND METHODS

Turtles were collected from 13 North Carolina counties between June 1982 and August 1989. Collecting was done primarily on the

¹Present address: Department of Biology, University of North Carolina at Asheville, Asheville, NC 28804.

Piedmont Plateau ($n = 97$), with small comparative samples taken from the Blue Ridge Mountains ($n = 16$) and the Coastal Plain ($n = 4$). Ninety-seven turtles were collected either as roadkills or while they were crossing highways. Twenty specimens were collected in the field, in part with the aid of a border terrier dog trained to locate turtles by scent. All turtles were sexed by secondary sexual characteristics or grouped as juveniles if the plastron length was <100 mm. The turtles were weighed to the nearest 0.1 g, measured both along the straight length of the plastron and around the curve of the carapace, and examined for helminth parasites. The entire visceral mass was removed. The body cavity and each organ was examined separately. The gastrointestinal tract was separated into distinct sections (esophagus, stomach, intestines, and colon), and individual sections were cut lengthwise, washed, and examined separately. After removal of parasites from the lumen, each section was scraped with a sharp blade. The gut sections and the contents were digested in a pepsin-HCl solution agitated constantly for 1 hour at 36C. The solution was decanted, and the residue was examined for helminths with the aid of a stereoscopic microscope.

Stomach ulcerations were removed and fixed in 70% ethyl alcohol, 5% formalin, or glutaraldehyde before sectioning for histological analysis. Blood smears were strained with hemal blood film stain and examined for the presence of microfilariae.

Helminths were considered prominent if the prevalence was $>15\%$ and peripheral if the prevalence was $<15\%$. We used an ANOVA to test for significant differences in number of species of parasites between sexes. A Kruskal-Willis test was used to test for significant differences in number of individual parasites between sexes and geographical regions of the state.

RESULTS

The box turtles we examined consisted of 43 males, 48 females, and 26 juveniles. Stuart and Miller (1987) previously reported on mass, sex and age structure, seasonal distribution, reproduction, and food habits of 104 individuals from this collection. Of the 117 turtles, 3 were not infected, 27 were infected with 1 species of helminth parasite, 41 were infected with 2 species, and 46 were infected with >3 parasite species. The modal number of helminths was 22 with a range of 0–303, exclusive of larval *Spironoura affinis* in stomach ulcers. The helminth species we found and their prevalence and intensity are shown in Table 1. No significant differences were found in helminth species prevalence or intensity between host males, females, and juveniles. Four helminth species exhibited a prominent infection rate of $>15\%$:

Table 1. Helminths collected from 117 turtles from North Carolina.

Parasite	Infection				
	Number	Percent (%)	Range	\bar{x}	SE
Platyhelminthes: Digenea					
Brachycoeliidae					
<i>Brachycoelium salamandrae</i>	3/117	2.6	7-9	8.3	0.67
Telorchidae					
<i>Telorchis robustus</i>	35/117	30	1-295	37.5	10.8
Nematoda:					
Molineidae					
<i>Oswaldocruzia</i> sp.	97/117	83	1-38	7.3	0.73
Cosmocercoididae					
<i>Cosmocercoides dukae</i>	24/117	20.5	1-241	22.3	10.4
<i>Aplectana</i> sp.	7/117	6	1-15	4.0	1.8
Kathlaniidae					
<i>Spironoura affinis</i>	89/117	76	1-151	32.0	4.0
Camallanidae					
<i>Serpinema microcephalus</i>	1/117	0.9			
Physalopteridae					
<i>Physaloptera</i> sp.	3/117	2.6	1-3	2.67	.88
Acanthocephala					
Oligacanthorhynchidae					
<i>Macracanthorhynchus ingens</i>	1/117	0.9			

Oswaldocruzia sp. (82.9%), *Spironoura affinis* Leidy, 1856 (76.1%), *Telorchis robustus* Goldberger, 1991 (29.9%), and *Cosmocercoides dukae* (Holl, 1928) (20.5%). An additional five species were considered peripheral with a prevalence of <15%. No extraintestinal helminths or microfilariae were found.

We found a morphologically distinct and unnamed species of *Oswaldocruzia* in the stomach of box turtles. Spicular morphology differs substantially from *O. pipiens* Walton, 1929 from amphibian hosts. Both the spicules and the dorsal ray of males are substantially larger than those of *O. pipiens*, although the species in the box turtles is smaller in all other respects. The prevalence of infection was 82.9% (97/117), making this the most common helminth parasite encountered. The mean intensity of infection was 7.3 worms per infected turtle with no significant difference between any age or sex classes ($P = 0.30$).

Spironoura affinis infected 76.1% (89/117) with a mean intensity of 32 nematodes per turtle. The range in intensity was 1-151. The difference in intensity of infection with *S. affinis* was nearly significant between adults and juveniles ($P = .056$); means for males were 20 ± 4.0 (SE), females 17 ± 4.6 , and juveniles 34.7 ± 8.4 . Pairwise contrasts

using a Mann-Whitney U-test between males, females, and juveniles indicated that the number of *S. affinis* in juveniles differed significantly from that in females ($P = 0.02$) but that there was no significant difference between males and females ($P = 0.26$), or between juveniles and males ($P = 0.13$). Fifty-two (44%) of the 117 turtles had active ulcer-like lesions in the fundal region of the stomach. Macroscopically, the lesions or ulcers showed a raised area 1–2 cm in diameter with a central opening 4–5 mm wide that extended into the stomach wall. Microscopically, the lesions showed a moderate to dense lymphoid infiltrate into the granulomatous lining of the ulcer. When pressure was applied to the base of these ulcers, masses of larval nematodes were expressed. Comparison with adult and immature nematodes already collected from the colon, particularly in regard to the shape of the esophageal bulb and the developing lip structures, showed these larvae to be *Spironoura affinis*. Many of the turtles had healed from previous ulcers, which suggests that the damage is tolerated.

In our survey, 29.9% of the turtles (35/117) were parasitized by *Telorchis robustus*, including 13 males, 15 females, and 7 juveniles. The number of worms per turtle was substantial with a mean (and range) of 62.2 (1–295) for males, 22.5 (1–93) for females, and 27.5 (1–80) for juvenile turtles. *Telorchis robustus* caused the only serious health problem seen in our study. One turtle with 223 worms had a partially telescoped intestine, apparently caused by the large worm mass.

Two of the male turtles hosted nine and seven *Brachycoelium salamandrae* (Froelich, 1789), respectively. One female harbored nine worms. None was found in juvenile turtles. The total prevalence was 2.6% (3/117) with a mean of 8.3 worms per turtle.

Twenty-four of the box turtles were infected with *Cosmocercoides dukae* (range = 1–241, \bar{x} = 22.3). The genus *Aplectana* is closely related to *Cosmocercoides* and is usually distinguished from the latter by the absence of plectanes in the male. Both are normally parasites of amphibians. Five (4.3%) turtles were infected with 1–15 sexually mature nematodes, lacking plectanes on the males. Because of the absence of plectanes, these worms were tentatively identified as *Aplectana* sp., but additional work on the morphology and life cycle is needed before a firm identification of the species can be made.

We also found two genera of spirurid nematodes. *Serpinema* (= *Camallanus*) *microcephalus* (Dujardin, 1845) was in the stomach of one turtle. This host was partially buried in the mud in a pool in an intermittent stream. The turtle had possibly swallowed infected copepods. Three turtle stomachs contained *Physaloptera* sp. Only two males were recovered. Based on the small sample size, we could not determine

whether these specimens were the species described from *Terrapene ornata* by Hill (1941) as *P. terrapenis* Hill, 1941.

One turtle contained one immature specimen of the acanthocephalan *Macracanthorhynchus ingens* (Linstow, 1879). This worm was not attached to the stomach wall and was possibly a spurious parasite contracted by the turtle having recently eaten an infected beetle.

DISCUSSION

The life histories of most of the parasites collected in this study are poorly documented, and much of the literature is either contradictory or limited in scope. In addition, major disagreement exists concerning the appropriate nomenclature for many species or species complexes. To help clarify existing information and to place our results in perspective for future studies, a summary of nomenclatural problems and life history data follows.

PLATYHELMINTHES: DIGenea

Brachycoelium salamandrae (Froelich, 1789)—Both Harwood (1932) and Byrd (1937) described a number of species of *Brachycoelium* from reptiles and amphibians in the southeastern United States. Rankin (1938) reviewed the genus and concluded that the characters used to describe the various species were too variable to be of specific diagnostic value. Rankin (1945) also stated that the relative size of individual flukes was dependent on the number of flukes infecting a particular host. The worms were quite small when large numbers were present but were substantially larger when ≤ 20 were present. Rankin advocated that *Brachycoelium daviesi* Harwood, 1932; *B. dorsale* Byrd, 1937; *B. georgianum* Byrd, 1937; *B. hospitale* Stafford, 1900; *B. louisianae* Byrd, 1937; *B. meridionalis* Harwood, 1932; *B. mesorchium* Byrd, 1937; *B. obesum* Nicoll, 1914; *B. ovale* Byrd, 1937; *B. storeriae* Harwood, 1932; and *B. trituri* Holl, 1928 be reduced to synonyms of *B. salamandrae*. Cheng (1958) disagreed, recognized all of the above listed species, and described a new species, *Brachycoelium elongatum* Cheng, 1958. Since that time, two additional species have been described: *B. stablefordi* Cheng and Chase, 1961 and *B. ambystomae* Couch, 1966. All specimens found in the box turtles in our study were identified with Cheng's keys as *Brachycoelium salamandrae*. Here, we follow Rankin in treating this species complex as a single, extremely variable species whose morphological features are influenced by numbers and hosts.

Brachycoelium salamandrae has been reported from a wide range of reptile and amphibian species. Rumbold (1928) reported *B. salamandrae* as the only trematode he found in seven box turtles from North Carolina, with an infection rate of 28% and an average of 0.28 worms

per turtle. Raush (1947) examined 19 box turtles in Ohio and found one turtle to host 27 specimens of *B. salamandrae*. Rankin (1945) listed the species distribution as worldwide, but Yamaguti (1971) listed only Palearctic and Nearctic hosts. Rankin (1945) noted a correlation between the terrestrial habits of certain amphibian hosts and a high level of prevalence, suggesting that terrestrial invertebrates were probably involved in transmission. Denton (1962) reported snails and slugs, *Praticollega berlandieriana* (Moricand), *Deroceras reticulatum* (= *Agriolimax agrestis*) (Mier), and *Mesodon thyroideus* (Say) as suitable experimental first intermediate hosts. Both motile and encysted cercariae were shed in secreted mucus. Uninfected *P. berlandieriana*, *D. reticulatum*, *Triodopsis texasiana* (= *Polygyra texasiana*) (Moricand), *Anguispira alternata* (Say), and *Bulimulus alternatus* (Say) became infected within 2–10 days after being exposed to infected first intermediate hosts, thus serving as second intermediate hosts. Jordan (1963) and Jordan and Byrd (1967) added *Triodopsis caroliensis* (Lea) and *Mesodon inflectus* (Say) to the list of first intermediate hosts and *T. caroliensis*, *M. inflectus*, *Zonitoides aboreus* (Say), *Gastrocopta contracta* (Say), *Stenotrema barbigerrum* (Redfield), *Philomycus carolianus* (Bosc), and *Deroceras laeve* (Müller) as second intermediate hosts. Cheng (1958) reported development of nonencysted metacercariae in *Ventridens ligera* (= *Zonitoides ligerus*) (Say). The definitive host presumably becomes infected by consuming snails with encysted metacercariae, because both Klimstra and Newsome (1960) and Stuart and Miller (1987) found that gastropods comprise a large percentage of box turtle diets. Given the broad range of first and second intermediate hosts that *B. salamandrae* is capable of infecting and the high frequency of these taxa in box turtle diets, it is surprising that the prevalence of infection is so low.

Telorchis robustus Goldberger, 1911—Wharton (1940) redefined the genus *Telorchis* and its species. We used his species key to identify *Telorchis robustus* from the box turtles in our study. Goldberger (1911) described *T. robustus* from a box turtle collected in Maryland, and Krull (1936) stated the trematode was common in Maryland box turtles. Bennett and Sharp (1938) found *T. robustus* in 38% (13/34) of *Terrapene c. triunguis* (Agassiz) examined in Louisiana. The number of worms ranged from three to nine, with an average of five worms per turtle. They also reported *T. robustus* from 12% (8/65) *Sternotherus odoratus* (Latreille) with an average infected of 10 worms per animal and a range of 1–28. Rausch (1947) reported *Telorchis* sp. from 1 of 19 box turtles in Ohio and *T. robustus* in four of eight *Clemmys guttata* (Schneider). The latter averaged two worms per turtle with a maximum of four. Thirteen of 35 turtles in our study had ≥ 25 worms

in the small intestines, which is substantially more than found in previous studies. The number of worms per turtle was substantial with a mean (and range) of 62.2 (1–295) for males, 22.5(1–93) for females, and 27.5 (1–80) for juvenile turtles. We do not know why the mean number of this trematode is so much higher in North Carolina box turtles than that reported from other localities or other species of turtles.

Krull (1935, 1936) reported that *Pseudosuccinea columella* (Say) became infected after eating trematode eggs (experimental infection) and began to shed xiphidiocercariae within 28–32 days. Cercariae successfully penetrated and encysted as metacercariae in three snail species: *P. columella*, *Helisoma trivolvis* (Say), and *Lymnaea traskii* (Lea). Krull postulated that turtles were infected during the spring and early summer when they ate snails in semi-flooded flats. He also noted that metacercariae were never abundant, although snails had been repeatedly exposed to thousands of cercariae. However, he did report that one snail would occasionally acquire a much heavier infection than others in the same group perhaps because they began feeding more quickly than others. In light of more recent studies on host immunity, genetic susceptibility might be a more reasonable cause than a behavioral trait. In either case, these “super-infected” snails will influence the range of worms in infected definitive hosts.

NEMATODA

Oswaldocruzia sp.—Seven species of *Oswaldocruzia* have been described from North American amphibians and reptiles (Baker 1977): *O. subauricularis* Travassos, 1917; *O. leidy* Travassos, 1917; *O. pipiens* Walton, 1929; *O. collaris* Walton, 1929; *O. waltoni* Ingles, 1936; *O. euryceae* Reiber, Byrd, and Parker, 1940; and *O. minuta* Walton, 1941. *Oswaldocruzia subauricularis* is a neotropical species and has only been reported once in the United States. Baker (1977) redescribed *O. pipiens* and regarded *O. collaris* and *O. eurycea* as synonyms of *O. pipiens*. He also treated *O. waltoni* and *O. minuta* as species inquirendae and *O. leidy* as a nomen nudum. Accounts of developmental and transmission patterns in *Oswaldocruzia* vary widely. Baer (1952) stated that *O. fillicollis* (Goeze) (presumably referring to *Oswaldocruzia filiformis* [Goeze, 1782] from amphibians molted twice within the egg and was thus infective when the egg was consumed. Baer also noted that L_3 larvae might sometimes hatch and remain ensheathed in the preceding molt until consumed. Baker (1978a) reported that eggs from frogs and toads were laid in the 16–cell stage, and L_1 larvae hatched within 24 hours of passage in the host's feces. Laboratory cultured specimens developed to the ensheathed, infective L_3 stage within 3–4 days at room temperature. Anuran infection occurs via

skin penetration. Larvae attached initially to the stomach mucosa but migrated posteriorly to the intestine as they matured. Baker felt that late summer and early fall, when marsh size was reduced and frog density was highest, was the most important period for parasite transmission and that *O. pipiens* could overwinter in its host. Hendrixx (1981) studied the seasonal fluctuation of *O. filliformis* in *Bufo bufo* L. in the Netherlands, and he reported L₄ larvae embedded in the stomach mucosa of overwintering hosts.

Oswaldocruzia specimens were only rarely found outside the stomach in the box turtles of our study. An infective mode involving skin penetration, while feasible in an amphibian, is somewhat more difficult to accept in the heavily armored box turtle. While softer areas of skin are found around the base of the legs and throat, chance nematode access and penetration could scarcely account for the very high levels of infection. The habits and habitats of the extremely wide range of amphibian and reptile hosts of *Oswaldocruzia* provide additional reasons to suspect an alternate route of infection. *Storeria dekayi* (Holbrook), *S. occipitamaculata* (Storer), *Anolis carolinensis* (Voigt), many ranid and bufonid species, *Typhlotriton spelaeus* Stejneger, and *Terrapene carolina* use a broad spectrum of habitats including semi-fossorial, arboreal, aquatic, semi-aquatic, cave-dwelling, and terrestrial. These habitat differences alone would severely hamper transmission of a parasite dependent on skin penetration to infect. Circumstantial evidence based on host diversity and host diet suggests that *Oswaldocruzia* might use an alternate life cycle with gastropods as intermediate hosts. In support of this contention, we found a small male *Oswaldocruzia* completely embedded in a piece of snail tissue taken from the stomach of a freshly killed box turtle. While as yet unproven, morphological and biological differences in the parasites and the different hosts suggest that the species of *Oswaldocruzia* in box turtles is distinct from *O. pipiens*.

Spironoura affinis Leidy, 1856—Leidy (1856) erected the genus *Spironoura* and listed two species: *S. gracile* from the stomach of the red-bellied turtle, *Pseudemys rubriventris* (Le Conte) (= *Emys serata*) and *S. affine*, later modified to *S. affinis* by Yamaguti (1961), from the cecum of the box turtle, *Terrapene carolina* (= *Cistudo carolina*). Yorke and Maplestone (1926) designated *S. gracile* as the type species apparently because it appeared first in Leidy's manuscript. This species has not, however, been collected since its description. Freitas and Lent (1942) felt that *S. gracile* should be considered a species inquirendum since Leidy's description was brief and incomplete. They proposed revalidation of the genus *Falcaustra* Lane, 1915 with all of the species of *Spironoura* transferred to this genus. Some authorities (Yamaguti

1961, Skrjabin et al. 1964) rejected this, but Chabaud (1978) considered *Falcaustra* to be the valid genus for this group. We follow Chapin (1924) in rejecting *Falcaustra* and continue to use *Spironoura* because Leidy's original description, although brief by today's standards, is sufficient to distinguish the genus, giving *Spironoura* priority.

About 50 species of *Spironoura* have been described from fishes, reptiles, and amphibians worldwide (Skrjabin et al. 1964). Mackin (1936) published a thorough study of the anatomy of the genus *Spironoura* and a key to the species from the United States. Five species of *Spironoura* have been reported from *Terrapene carolina* in the United States: *Spironoura affinis* Leidy, 1856; *S. longispicula* (Walton, 1927); *S. cryptobranchi* Walton, 1930; *S. chelydrae* (Harwood, 1930); and *S. concinnae* Mackin, 1936. Canavan (1929) described *S. procera* from the same host, *Pseudemys rubriventris* (= *Emys serata*), and from the same locality where Leidy worked, i.e., Philadelphia. Harwood (1930) is the only other investigator to have reported the presence of *Spironoura procera*, but he said that it was not sufficiently distinct from *S. affinis* to merit specific status. Harwood's (1932) work, plus our own study of *S. affinis* and examination of the specimen from the U.S. National Museum Helminthological Collection, Beltsville, Maryland marked "*Spironoura procera*? (No. 52145)," suggest that *S. affinis*, *S. procera*, and *S. gracile* are all members of the same species. We also borrowed specimens identified as *S. concinnae*, collected from *Terrapene carolina* in Mississippi, from the U.S. National Museum Helminthological Collection (No. 66152). Comparison with specimens of *S. affinis* collected from the same host in North Carolina convinced us that Caballero (1939) was correct in considering *S. concinnae* as a synonym of *S. affinis*. We have followed Yamaguti's (1961) designation of *Spironoura affinis* to minimize confusion. However, this diverse and complex genus needs revision.

Cosmocercoides dukae (Holl, 1928)—*Cosmocercoides dukae* is a common parasite of amphibians. Holl (1928) originally described *C. dukae* (= *Cosmocerca dukae*) from a newt collected in Durham, North Carolina. Harwood (1932), apparently unaware of Holl's work, described the same species as *Oxysomatium variabilis* and listed 10 species of amphibian and reptile hosts. Harwood's experimental attempts to demonstrate host infection by skin penetration were not successful, but he felt that a direct life cycle was probable. Ogren (1953, 1959) demonstrated the nematode's ability to complete its life cycle in a variety of gastropod species, including *Ashmunella rhyssa* (Dall), *Triodopsis* (= *Polygyra*) *fosteri* (Baker), *Retinella* sp. Fischer, and *Deroceras* sp. Rafinesque. Anderson (1960) described the life cycle of *C. dukae* in *Discus cronkhitei* (Newcombe), *Zonitoides aboreus* (Say), and *Deroceras*

Table 2. Helminths reported from *Terrapene carolina*.

Parasite	Number Infected/Examined	Locality	Reference
MONOGENEA			
Polystomidae			
<i>Neopolystoma terrapenis</i> (Harwood, 1932)	1/14	Tex.	Harwood (1932)
<i>Polystomoidella oblongum</i> (Wright, 1879)	1/16	La.	Acholonu (1969)
<i>P. whartoni</i> (Price, 1939)	1/16	La.	Acholonu (1969)
<i>Polystomoides coronatum</i> (Leidy, 1888)			Braun (1890) (from Ernst and Ernst 1977)
DIGENEA			
Brachycoeliidae			
<i>Brachycoelium salamandrae</i> (Froelich, 1789)	2/7	N.C.	Rumbold (1928)
	1/19	Oh.	Rausch (1947)
Telorchidae			
<i>Telorchis</i> sp.	1/19	Oh.	Rausch (1947)
<i>T. corti</i> Stunkard, 1915	2/63	Ill.	Martin (1973)
<i>T. robustus</i> Goldberger, 1911	common	Md.	Goldberger (1911); Krull (1935, 1936)
	13/34	La.	Bennett and Sharp (1938)
CESTOIDEA			
Protocephalidae			
<i>Proteocephalus</i> sp.	1/14	Tex.	Harwood (1932)
Anoplocephalidae			
<i>Oochoristica whitentoni</i> Steelman, 1939	1/12	Ok.	Steelman (1939)
immature cyclophyllidean	1/34	La.	Bennett and Sharp (1938)
NEMATODA			
Dipetalonematidae			
<i>Cardianema cistudinis</i> (Leidy, 1856)		Penn.?	Leidy (1856)
		D.C.	Alicata (1933)
	1/4	La.	Herban and Yeager (1969)
Atractidae			
<i>Atractis carolinae</i> Harwood, 1932	13/14	Tex.	Harwood (1932)

Table 2. Continued.

	Number Infected/Examined	Locality	Reference
Cosmocercidae			
<i>Aplectana</i> sp.	7/7	N.C.	Rumbold (1928)
	1/19	Oh.	Rausch (1947)
	12/16	La.	Acholonu and Amy (1970)
<i>Cosmocercoides dukae</i> (Holl, 1928)	7/14	Tex.	Harwood (1930, 1932)
	1/19	Oh.	Rausch (1947)
Kathlaniidae			
<i>Cruzia testudines</i> Harwood, 1932	13/14	Tex.	Harwood (1932)
<i>Spironoura</i> sp. Leidy, 1856	11/63	Ill.	Martin (1973)
		Md.	Ernst and Ernst (1975)
<i>S. affinis</i> Leidy, 1856		Penn.	Leidy (1856)
			Boulenger (1923)
	13/14	Tex.	Harwood (1932)
	9/19	Oh.	Rausch (1947)
	6/63	Ill.	Martin (1973)
<i>S. chelydrae</i> Harwood, 1932	4/5	Tenn.	Limsuwan and Dunn (1978)
<i>S. cryptobranchi</i> Walton, 1930		La.	Bennett and Sharp (1938)
<i>S. longispiculata</i> Walton, 1927		Penn.?	Walton (1927)
Molineidae			
<i>Oswaldocruzia leidy</i> Travassos, 1917		Penn.?	Leidy (1856)
		La.?	Steiner (1924)
	2/7	N.C.	Rumbold (1928)
	10/19	Oh.	Rausch (1947)
	3/16	La.	Acholonu and Amy (1970)
		Md.	Ernst and Ernst (1975)
<i>O. pipiens</i> Walton, 1929	2/14	Tex.	Harwood (1932)
<i>O. leidy</i> & <i>O. pipiens</i>	10/63	Ill.	Martin (1973)
Camallanidae			
<i>Serpinema microcephalus</i> (Dujardin, 1845)	2/16	La.	Acholonu and Amy (1970)
	7/63	Ill.	Martin (1973)

Table 2. Continued.

	Number Infected/Examined	Locality	Reference
Physalopteridae			
<i>Physaloptera terrapenis</i> Hill, 1941	7/47 in	Ok.	Hill (1941)
<i>T. ornata</i>			
Spiruridae			
<i>Spiroxys constricta</i> (Leidy, 1856)	7/16	La.	Acholonu and Amy (1970)
		Penn.?	Leidy (1856)
<i>S. contorta</i> (Rudolphi, 1819)	11/63	Ill.	Martin (1973)
Gnathostomatidae			
<i>Gnathostoma procyonis</i> Chandler, 1942	3/4	La.	Ash (1962)

laeve (Müller) (= *D. gracile* Rafinesque) and suggested that amphibian infections occurred from ingestion of infected molluscs. Baker (1978b) reported that *C. dukae* larvae burrow through the skin of toads and migrate through the body. This versatility in ability to use such different definitive hosts suggested to Baker that *C. dukae* represented an early stage of parasitic adaptation.

PARASITES AND HOST DIET

Surveys from various areas within the range of *Terrapene carolina* indicate that a broad range of helminth parasites infect the box turtle (Table 2). In North Carolina, parasite presence appears to be regulated primarily by diet. Recognizable items found in the gastrointestinal tract of 72 turtles, in order of frequency of occurrence, were snails (Gastropoda) - 59%, insects (Insecta) - 43%, sowbugs (Isopoda) - 40%, plant material (primarily fungi) - 32%, slugs (Gastropoda) - 7%, rodents (Mammalia) - 5.5%, earthworms (Oligochaeta) - 3%, and millipeds (Diplopoda) - 3%. *Cosmocercoides dukae*, *Brachycoelium* spp., and *Telorchis robustus* have all been shown to use molluscan intermediate hosts (Krull 1935, 1936; Ogren 1953; Jordan and Byrd 1967). Our evidence strongly suggests that *Spironora affinis* and *Oswaldocruzia* sp. also might be capable of using molluscs to reach the definitive host. The records for *Serpinema microcephallus* and *Physaloptera* sp. probably represent rare or accidental infections. The life cycle of most physalopterans is unknown; those that are known use invertebrate intermediate hosts. The specimen of *Macroacanthorhynchus ingens* indicates recent ingestion of a scarabid beetle. Considering the relatively high incidence of insects and sowbugs in the diet and the semi-aquatic nature of the turtles, the absence of acanthocephalans is some-

what surprising. The poisonous fungi in the diet could conceivably act as a periodic vermifuge, but that has not been investigated.

Parasite life cycles are often complex and may involve a variety of strategies to get the parasite into the definitive host, including one or more intermediate hosts. Successful transmission requires a congruence between parasite life cycle and host behavior or ecology. Aho (1990) discussed the importance of reptile- and amphibian-parasite systems in understanding the ecological and evolutionary relationships which determine parasite species distribution. The parasite presence/absence data accumulated in our study suggest that *Spironoura affinis* has a long history or relationship with *Terrapene carolina* and the aquatic emydid turtles from which the box turtle evolved. Nursery ulcers and molluscan intermediate hosts could represent the nematode's adaptation to the host's move from an aquatic to a terrestrial habitat. The presence/absence data also suggest that *Terrapene carolina* acquired a number of amphibian parasites in the ecological shift from water to land.

ACKNOWLEDGMENTS—This study was supported in part by Science and Engineering Development Award 89SE01 from the North Carolina Board of Science and Technology. We are indebted to J. Ralph Lichtenfels for the loan of specimens from the U.S. National Museum Helminthological Collection. We further thank James W. Petranks for assistance with statistical analyses, John G. Petranks for updating the molluscan nomenclature, and to Joyce McHenry, Jeanne Simmons, and Kathy Haley for assistance in collecting specimens.

LITERATURE CITED

- Acholonu, A. D. 1969. Some monogenetic trematodes from Louisiana turtles. *Proceedings of the Louisiana Academy of Sciences* 32:20–25.
- Acholonu, A. D., and K. Arny. 1970. Incidence of nematode parasites in Louisiana turtles. *Proceedings of the Louisiana Academy of Sciences* 33:25–34.
- Aho, J. M. 1990. Helminth communities of amphibians and reptiles: comparative approaches to understanding patterns and processes. Pages 157–195 in *Parasite communities: patterns and processes*. (G. Esch, A. Bush, and J. Aho, editors) Chapman and Hall, New York, New York.
- Alicata, J. E. 1933. A new genus for the nematode *Filaria cistudinis* Leidy, 1856, of the family Filaridae. *Journal of the Washington Academy of Sciences* 23:95–100.
- Anderson, R. C. 1960. On the development and transmission of *Cosmocercoides dukae* of terrestrial molluscs in Ontario. *Canadian Journal of Zoology* 38:801–825.

- Ash, L. R. 1962. Development of *Gnathostoma procyonis* Chandler, 1942 in the first and second intermediate hosts. *Journal of Parasitology* 48:298-305.
- Baer, J. G. 1952. Ecology of animal parasites. University of Illinois Press, Urbana.
- Baker, M. R. 1977. Redescription of *Oswaldocruzia pipiens* Walton, 1929 (Nematoda: Trichostrongylidae) from amphibians of eastern North America. *Canadian Journal of Zoology* 55:104-109.
- Baker, M. R. 1978a. Development and transmission of *Oswaldocruzia pipiens* Walton, 1929 (Nematode: Trichostrongylidae) in amphibians. *Canadian Journal of Zoology* 56:1026-1031.
- Baker, M. R. 1978b. Transmission of *Cosmocercoides dukae* (Nematoda: Cosmocercidae) to amphibians. *Journal of Parasitology* 64:765-766.
- Bennett, H. J., and C. H. Sharp. 1938. Helminth parasites of *Sternotherus odoratus* and *Terrapene carolina triunguis* from Louisiana. *Proceedings of the Louisiana Academy of Sciences* 4:241-242.
- Boulenger, C. L. 1923. A nematode (*Falcaustra chapini* n. sp.) parasitic in a North American tortoise. *Parasitology* 15:49-53.
- Byrd, E. E. 1937. Observations on the trematode genus *Brachycoelium* Dujardin. *Proceedings of the United States National Museum* 84:183-199.
- Caballero, E. 1939. Nematodos de los reptiles de Mexico. V. *Anales del Instituto de Biologia* 10:275-282.
- Canavana, W. P. N. 1929. Nematode parasites of vertebrates in the Philadelphia Zoological Garden and vicinity. *Parasitology* 21:63-102.
- Chabaud, A. G. 1978. Keys to genera of the superfamilies Cosmocercidae, Seuratiodea, Heterakoidea and Subuluroidea. Pages 1-71 in CIH keys to the nematode parasites of vertebrates. Number 6 (R. Anderson, A. Chabaud, and S. Willmott, editors) Commonwealth Agricultural Bureaux, Farnham Royal, Bucks, England.
- Chapin, E. A. 1924. Note on *Spironoura affine* Leidy, 1856. *Journal of Parasitology* 10:212.
- Cheng, T. C. 1958. Studies on the trematode family Dicrocoeliidae 1. The genera *Brachycoelium* (Dujardin, 1845) and *Leptophallus* Luhe, 1909 (Brachycoeliinae). *American Midland Naturalist* 59:67-81.
- Denton, J. F. 1962. The life cycle of *Brachycoelium mesorchium* Byrd, 1937. *Journal of Parasitology* 48 (Sec. 2):22.
- Ernst, E. M., and C. H. Ernst. 1975. New hosts and localities for turtle helminths. *Proceedings of the Helminthological Society of Washington* 42:176-178.
- Ernst, E. M., and C. H. Ernst. 1977. Synopsis of helminths endoparasitic in native turtles of the United States. *Bulletin of the Maryland Herpetological Society* 13:1-75.
- Freitas, F. T., and H. Lent. 1942. Contribuição ao conhecimento da subfamília Kathlaniinae Lane, 1914 (Nematoda, Subuluroidea). *Arquivos de Zoologia* (Sao Paulo) 3:13-41.

- Goldberger, J. 1911. On some new parasitic trematode worms of the genus *Telorchis*. Bulletin of the Hygiene Laboratory of the United States Public Health and Marine Hospital Service 71:36-47.
- Harwood, P. D. 1930. A new species of *Oxysomatium* (Nematoda) with some remarks on the genera *Oxysomatium* and *Aplectana*, and observations on the life history. Journal of Parasitology 17:61-73.
- Harwood, P. D. 1932. The helminths parasitic in the Amphibia and Reptilia of Houston, Texas, and vicinity. Proceedings of the United States National Museum 81:1-71.
- Hendriks, W. M. L. 1981. Seasonal fluctuation localization and inhibition of development of parasitic stages of *Oswaldocruzia filiformis* (Goeze, 1782) Travassos, 1917 (Nematoda: Trichostrongylidae) in *Bufo bufo* L., 1785 under natural and experimental conditions in the Netherlands. Netherlands Journal of Zoology 31:481-503.
- Herban, N. L., and Yaeger, R. G. 1969. Blood parasites of certain Louisiana reptiles and amphibians. American Midland Naturalist 82:600-601.
- Hill, W. C. O. 1941. *Physaloptera terrapenis*, a new nematode from a tortoise. Transactions of the American Microscopical Society 60:59-64.
- Holl, F. J. 1928. Two new nematode parasites. Journal of the Elisha Mitchell Scientific Society 43:184-186.
- Jordan, H. E. 1963. Studies on the life history of *Brachycoelium mesorchium* Byrd, 1937 (Trematoda: Brachycoeliinae). Dissertation Abstracts 23:4472-4473.
- Jordan, H. E., and E. E. Byrd. 1967. The developmental cycle of *Brachycoelium mesorchium* Byrd, 1937 (Trematoda: Brachycoeliinae). Transactions of the American Microscopical Society 86:67.
- Klimstra, W. D., and F. Newsome. 1960. Some observations on the food coactions of the common box turtle, *Terrapene c. carolina*. Ecology 41:639-647.
- Krull, W. H. 1935. A note on the life history of *Telorchis robustus* Gold. (Trematoda: Telorchidae). Proceedings of the Helminthological Society of Washington 2:65.
- Krull, W. H. 1936. Studies on the life history of *Telorchis robustus* (Trematoda: Plagiorchiidae). Proceedings of the Helminthological Society of Washington 4:54-56.
- Leidy, J. 1856. A synopsis of Entozoa and some of their ectocongeners observed by the author. Proceedings of the Academy of Natural Sciences of Philadelphia 8:42-58.
- Limsuwan, C., and M. C. Dunn. 1978. A survey of helminth parasites from turtles in Rutherford County, Tennessee. Journal of the Tennessee Academy of Sciences 53:111-114.
- Mackin, J. G. 1936. Studies on the morphology and life history of nematodes in the genus *Spironoura*. Illinois Biological Monographs 14(3):1-64.
- Martin, D. R. 1973. Distribution of helminth parasites in turtles native to southern Illinois. Transactions of the Illinois Academy of Sciences 65:61-67.

- Ogren, R. E. 1953. A contribution to the life cycle of *Cosmocercoides* in the snails (Nematoda: Cosmocercidae). Transactions of the American Microscopical Society 72:87-91.
- Ogren, R. E. 1959. The nematode *Cosmocercoides dukae* as a parasite of the slug. Proceedings of the Pennsylvania Academy of Sciences 33:236-241.
- Rankin, J. S. 1938. Studies on the trematode genus *Brachycoelium* Duj. I. Variation in specific characters with reference to the validity of the described species. Transactions of the American Microscopical Society 57:358-375.
- Rankin, J. S. 1945. An ecological study of the helminth parasites of amphibians and reptiles of western Massachusetts and vicinity. Journal of Parasitology 31:142-150.
- Rausch, R. 1947. Observations on some helminth parasitic in Ohio turtles. American Midland Naturalist 38:434-442.
- Rumbold, D. W. 1928. The ecology of the helminth parasites of Testudinata. Ph.D. Thesis, Duke University, Durham, North Carolina.
- Skrjabin, K. I., N. P. Shikhobalova, and E. A. Lagodovskaya. 1964. Oxyurata of animals and man. Part Three. Academy of Sciences of the USSR, Helminthological Laboratory, Moscow. (Translated by the Israel Program for Scientific Translations, Jerusalem. 1976).
- Steelman, G. M. 1939. *Oochoristica whitentoni*, a new anaplocephalid cestode from a land tortoise. Journal of Parasitology 25:479-482.
- Steiner, G. M. 1924. Some nemas from the alimentary tract of the Carolina tree frog (*Hyla carolinensis* Pennant) Journal of Parasitology 11:1-32.
- Stuart, M. D., and G. C. Miller. 1987. The eastern box turtle, *Terrapene c. carolina* (Testudines: Emydidae) in North Carolina. Brimleyana 13:123-131.
- Walton, A. C. 1927. A revision of the nematodes of the Leidy collection. Proceedings of the Academy of Natural Sciences of Philadelphia 79:49-163.
- Wharton, G. W. 1940. The genera *Telorchis*, *Protenes*, and *Auridistomum* (Trematoda: Reniferidae). Journal of Parasitology 26:497-518.
- Yamaguti, S. 1961. Systema helminthum. The nematodes of vertebrates. Part I and II, Volume III. Interscience Publishers, New York, New York.
- Yamaguti, S. 1971. Synopsis of digenetic trematodes of vertebrates. 2 Volumes. Keigaku Publishing Company, Tokyo.
- Yorke, W., and P. A. Maplestone. 1926. The nematode parasites of vertebrates. J. & A. Churchill, London.

Accepted 4 May 1992